CAUSES OF STERILITY IN WHEAT

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Reproductive development in wheat

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Summary

This paper provides an introduction to the complex changes that occur during reproduction in the wheat plant, and is mainly intended for field scientists who are unfamiliar with events that occur at the apical meristem. Growth stages during reproductive development in wheat are identified and briefly described. In addition, stages of reproductive development that are likely to be particularly vulnerable to environmental stresses are suggested.

Introduction

It is convenient to divide the life cycle of wheat into developmental stages, the simplest being vegetative and reproductive growth. Recognizable events during vegetative growth include leaf initiation, root initiation, tillering and stem elongation. During vegetative growth, the shoot apex (Williams 1966) is wrapped in its subtending leaves close to the base of the plant. There, the apical meristem will undergo profound developmental changes (Barnard 1955), after evocation, to form the complex reproductive spike. Only then is there substantial stem growth: the spike is pushed upwards as a result of intercalary growth of the stem internodes to emerge from the flag leaf.

Many workers have codified stages in cereal development or have provided brief descriptions. The most often used are those of Feekes (Large 1954) and Zadoks (Zadoks *et al.* 1974, Tottman and Makepeace 1979). The Zadoks growth scale (Table 1) has the advantage that it includes both the external and dissected appearance of the plant and covers growth from germination to seed maturity. Harrell *et al.* (1993) give a computer program which converts between a number of developmental scales, including Zadoks and Feekes. Useful illustrations of wheat development are contained in Kirby and Appleyard (1987), Perry and Hillman (1991). This review focusses on developmental events from the transition of the apical meristem to grain maturation. It identifies key references which should be sought for more detailed information.

Table 1. Development of the wheat plant: the ten principal decimal codes of the Zadoks growth scale and their descriptors as defined by Zadoks *et al.* (1974).

Code	Growth stage
0	• germination
1	• seedling growth (no. of unfolded leaves on the main shoot)
2	• tillering
3	• stem elongation (no. of detectable nodes)
4	• booting
5	• ear emergence
6	• anthesis
7	• milk development
8	• dough development
9	• ripening

Floral morphology

Before describing the ontogony of the inflorescence, it is necessary to become familiar with the morphology and terminology of the spikelet and flower (Figure 1). The inflorescence is a spike, with the main axis, the rachis, consisting of short internodes bearing the sessile spikelets alternately on opposite sides (1-3 basal spikelets are often rudimentary) and a terminal spikelet. Each spikelet consists of a central axis, the rachilla, carrying two rows of alternate florets, subtended by two basal bracts (empty glumes). The spikelet bears 2-5 perfect lower florets and 3-8 imperfect upper florets. Each perfect floret has a pistil, 3 stamens, 2 small scales (lodicules = reduced perianth), all enclosed by 2 flowering glumes (palea + lemma). The ovary has one cell, one ovule and 2 short style lobes each with a feathery stigma. The stamen consists of a filament and a bilobed anther, and each anther lobe contains two loculi.

Development of the inflorescence

The chronological relationship between development of the inflorescence and vegetative development are given by Langer and Hanif (1973), Kirby (1974), Baker and Gallagher (1983) and Pinthus (1985). Nerson *et al.* (1980) provides a useful sequence of descriptors for morphological events at the shoot apex (Table 2). Other documented sequences worth examining are those of Zadoks, referred to earlier, and Klepper *et al.* (1983). The following papers provide relevant illustrative material: Barnard (1955), Moncur (1981), Gardner *et al.* (1985), Kirby and Appleyard (1987), Sibony and Pinthus (1988).

Table 2. Developmental stages in the differentiation of the wheat spike as defined by Nerson *et al.* (1980).

Stage	Description
0	• vegetative shoot apex
1	• apex begins to elongate
2	• elongation with single ridges
3	• spikelet primordia appear as double ridges
4	• spikelet primordia at the centre of the spike begin to swell
5	• most spikelet primordia are swollen
6	• spikelet differentiation begins, some glumes and floret initials become visible
7	• floret initials in most spikelets, some lemma primordia become visible
8	• terminal spikelet appears, pistil and stamen primordia visible in some florets
9	• terminal spikelet differentiated at right angles to other spikelets

Differentiation of the spike

For each cultivar there is a relationship between elongation of the shoot apex and the number of visible, emerged leaves on the stem. Elongation of the shoot apex precedes the onset of the development of the inflorescence. The first morphological evidence of floral initiation is the appearance of the double ridge consisting of the spikelet primordia and subtending leaf initials. The subtending leaf primordia normally cease growth early and rarely develop into more than a collar (Sharman 1983). The most advanced buds of the double ridges are near the middle of the spike. However, each succeeding spikelet bud develops faster than the one initiated before. The rate of differentiation increases acropetally and, by pre-anthesis, the central spikelets are the most developed.

Differentiation of the spikelet

Differentiation of the spikelet starts in the lower midpart of the spike and proceeds acropetally and basipetally. The number of florets is indefinite, however fewer than 4-8 normally develop. Sibony and Pinthus (1988) describe 10 stages of spikelet differentiation in spring wheat.

Differentiation of the floret
The primordium of the palea appears first followed by four protuberances (3 stamens, ovary), followed by the lodicules. Growth of the peduncle moves the ear upwards within the 'boot' formed by the flag leaf sheath - booting is followed by ear peep and by continued growth of the peduncle. Only some 30-40 % of florets set grain. It is generally agreed that grain set is restricted to florets which have distinct anther lobes at spike emergence (Sibony & Pinthus 1988). A greater understanding of factors controlling floret initiation and development is anticipated with refinement of *in vitro* sytems currently in use (Kovacs *et al.* 1993, Astwood and Hill 1995).

Development of the anther and pollen
The cardinal events during development of the pollen grain and the male gametophyte are given in Table 3. Meiosis occurs in the pollen mother cells when the anthers are green and about 1 mm in length. This occurs about 6-14 days prior to anthesis (approx. at the late boot stage) but varies with climatic conditions and cultivar. Meiosis is synchronous between the three anthers of a single floret. Following meiosis, the haploid cells of the tetrads are obvious with their common callose-rich walls. The young, highly vacuolated microspores separate and grow rapidly forming a large central vacuole. At the first mitosis, the microspores become pollen grains and rapidly differentiate into mature pollen grains. The latter stages are highly complex and require the synthesis and laying down of the multilayered pollen wall and nuclear divisions, as well as the accumulation of carbohydrate reserves, chiefly as starch. More information on specific aspects of late pollen development is contained in Stanley and Liskens (1974), Kress and Stone (1983), and El-Ghazaly and Jensen (1986a,b, 1987). Cheng and McComb (1992) were successful in germinating pollen grains of wheat in the laboratory.

Table 3. Key events in the development of pollen and the pollen tube in wheat.

Main events	Other events in the flower
• pollen mother cells (anther <1 mm in length and white/translucent)	• formation of the tapetum
• meiosis (anther ca. 1 mm in length and green)	• meiosis of the megaspore mother cell, differentiation of feathery stigmatic lobes
• pollen tetrad	
• microspores	
• mitosis	• lignification of the endothecium
• pollen grains with exine (anther ca. 3-5 mm in length and yellow)	• senescence of the tapetum
• starch accumulation	
	• elongation of the filament
• anthesis	• swelling of the lodicules, opening of the anther pore
• pollination	
• germination of pollen	• pollen - stigma interaction
• pollen tube growth through the style	• pollen tube - style interaction
• double fertilization	• pollen tube - embryo sac interaction

Anthesis

Although anthesis can commence at booting in some varieties, it usually occurs a few days after spike emergence (heading). Anthesis usually starts in the basal florets of the central spikelets and proceeds basipetally and acrpoetally within the spike and acropetally in the spikelets. In any one spike, anthesis is complete within 2-3 days. Anthesis of tillers follows that of the main shoot. Opening of florets is facilitated by the swelling of the lodicules which push apart the lemma and palea. This takes about 5 minutes during which time the filaments elongate rapidly, the anthers are pushed upwards and the lobes dehisce from the tips downward along the connection between the loculi. Florets are usually self-pollinated. The duration of opening can span from 6 to over 60 minutes. Unpollinated florets (in open flowering genotypes) remain open for several days. About 17-24 hours after transfer of pollen to the stigma, the stigma shrivels. Unlike pollen which has short viability (15-30 minutes) in air, the stigma can remain receptive for 4-6 days.

Development of the ovary

The female part of the wheat flower, the pistil, consists of the stigma (area to receive and screen the pollen grains), the style (through which the pollen tubes grow to reach the ovule) and the basal ovary

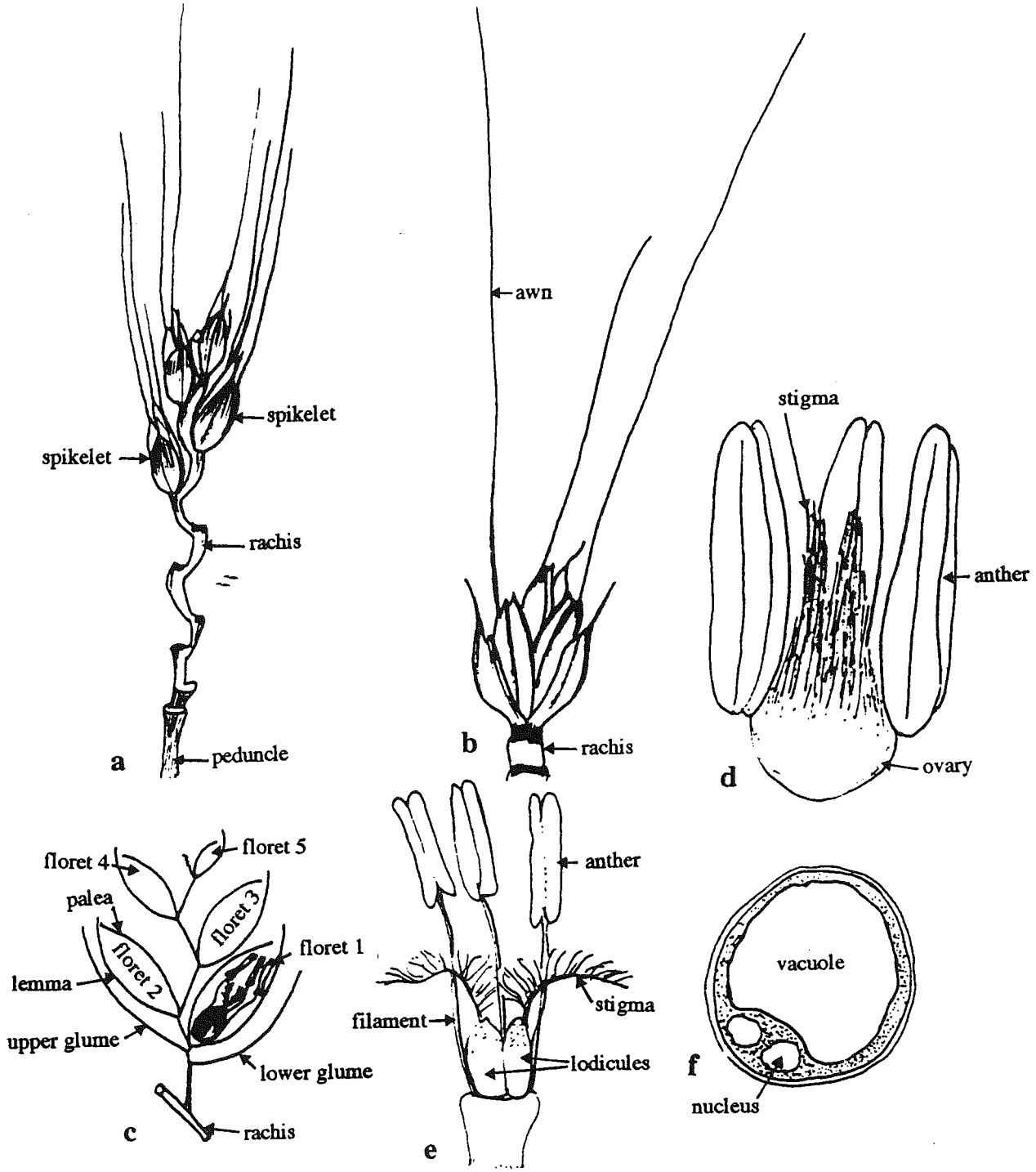

Figure 1. Structure of the wheat ear. **a.** Part of the ear with the basal spikelets removed to show the rachis and alternate insertion regions for the spikelets. **b.** Fully grown spikelet in face view. **c.** Diagram of a spikelet with 5 florets. **d.** Floret dissected to show green anthers 3 mm in length and the partially developed style. Pollen stage as illustrated in f. **e.** Floret dissected close to anthesis showing the elongated filaments and fully formed stigmas. **f.** Pollen grain at the two-nuclear, vacuolate stage prior to full exine formation and the accumulation of starch.

containing the single ovule. The embryo sac develops from the single megaspore mother cell after it has undergone meiosis. The papers by Bennet *et al.* (1973a,b) contain relevant information on meiosis and cell division. Pollen pistil interaction in wheat is reported by Vishnyakova and Willemse (1994). Following double fertilization, the embryo and endosperm (tissue which provides nourishment for the developing embryo and later for the seedling after germination) differentiate and grow. Recognizable growth stages as the ovary transforms into the mature grain, are given in Table 4.

Table 4. Developmental stages of the wheat grain defined using endosperm and embryo traits.

Endosperm development	Embryo + endosperm development [1]
• watery	• 1-5 DPA [2]: embryo globular, rapid endosperm growth, seeds are white-green and < 4 mm length • 5-10 DPA: embryo forms axis and scutellum, rapid endosperm growth
• milky	• 10-15 DPA: scutellum elongates, endosperm becomes milky • 15-20 DPA: primary leaf, root and coleoptile form, full seed size, seeds fresh green
• soft dough	• 20-30 DPA: soft dough endosperm, rapid increase in embryo dry weight, seeds partly yellow
• hard dough • ripe • dead ripe	• 30-50 DPA: seeds desiccate and turn brown (includes hard dough, ripe and dead ripe categories)

Notes: [1] After Noda *et al.* 1994, [2] DPA = days post anthesis.

Post-fertilization ovary growth is characterized by three phases:

- a short period of exponential growth of 10-14 days duration
- a period of 15-35 days duration of constant increase in dry weight as starch is deposited in the endosperm and the grain takes on a milk-like and then dough-like consistency
- a period of slower growth when waxy substances are deposited in vascular strands and maximum dry matter is reached (physiological maturity, Singh *et al.* 1984). The moisture content of the grain then declines rapidly to 10-20 %.

There is considerable information on endosperm formation, lack of direct vascular connections between the diploid tissues of the ovule and the polyploid endosperm, and the supply of nutrients to the developing grain. The following provide a brief introduction to the area: anatomy of the rachis (Whingwiri *et al.* 1981); endosperm morphogenesis (Evers 1970, Campbell *et al.* 1981, Smart and O'Brien 1983, Huber and Grabe 1987; rate of grain fill (Walpole and Morgan 1970, Bruckner and Frohberg 1987); and the redistribution of mineral nutrients to the grain (Hocking 1994). The pattern of grain set in ears is discussed by Evans *et al.* (1972) and more recent workers examining effects of environmental stresses on grain development.

Stress and reproduction

The potential impact of pre- and post anthesis stress on reproduction are considered in some detail by Rawson (this volume) and will not be discussed in detail here. Rather, I would like to draw the reader's attention to key processes that potentially could be impacted on by single or interactive environmentally imposed stresses (Table 5). Of particular interest is the potential for micro-nutrient deficiencies to affect male sporogenesis. Four elements have so far been implicated in causing male sterility (i.e. either abnormal pollen or impaired pollen viability) in cereals, namely copper (Graham 1975), boron (Rerkasem *et al.* 1989), manganese (Sharma *et al.* 1991) and molybdenum (Agarwala *et al.* 1979). However, the

Table 5. Processes during reproductive development that may be adversely affected by environmental stress.

Critical process impaired	Affected organ or cell	Possible environmental stress
• DNA replication (meiosis)	• pollen mother cells, megaspore mother cell	• heavy metals, high temperature, copper deficiency
• cell turgor	• floret initial, pollen mother cells, lodicule, endosperm,	• water deficit
• transpiration	• poorly transpiring apical organs	• high humidity, water deficit
• supply of photosynthate	• floret initials, tapetum, pollen, endosperm	• high or low temperatures, low radiation, nutrient deficiencies
• lignification and related pathways	• endothecium, xylem, pollen exine	• copper, manganese or boron deficiency
• protein synthesis	• endosperm	• nutrient deficiency, water deficit, low radiation

evidence is convincing only for copper and boron resulting in male sterility in field crops. It appears that meiosis in the anther is particularly sensitive to copper deficiency (Graham 1975, Azouaou and Souvre 1993), although other processes such as lignification of the endothecium (Dell 1981) and growth of the tapetum (Jewell *et al.* 1988) may also be affected. Rather less is known about the sensitivity of reproductive stages to low boron supply except that male structures are more severely affected than female parts (Chen and Rerkasem 1993, Rerkasem *et al.* 1993). Recent data (Dell and Rerkasem, unpublished) for two wheat cultivars, show that pollen development is impaired between separation of the microspores after meiosis and starch accumulation in boron deficient plants. Hence, boron deficiency within the week prior to anthesis is anticipated to have a major impact on grain set. How environmental factors, such as drought, low temperature and high humidity, interact with boron supply to the anther are largely unknown and require investigation in the field and in controlled growth chambers.

References

Agarwala, S.C., Chatterjee, C., Sharma, P.N., Sharma, C.P. and Nautiyal. 1979. Pollen development in maize plants subjected to molybdenum deficiency. Canadian Journal of Botany 57, 1946-1950.

Astwood, J.D. and Hill, R.D. 1995. Barley spikelet culture - an effective tool for the analysis of biochemical events in flower development. Plant Cell Tissue and Organ Culture 40, 93-96.

Azouaou, Z. and Souvre, A. 1993. Effects of copper deficiency on pollen fertility and nucleic acids in the durum wheat anther. Sexual Plant Reproduction 6, 199-204.

Baker, C.K. and Gallagher, J.N. 1983. The development of winter wheat in the field. 1. Relation between apical development and plant morphology within and between seasons. Journal of Agricultural Science, Cambridge 101, 327-335.

Barnard, C. 1955. Histogenesis of the inflorescence and flower of *Triticum aestivum* L. Australian Journal of Botany 3, 1-20.

Bennet, M.D., Finch, R.A., Smith, J.B. and Rao, M.K. 1973a. The time and duration of female meiosis in wheat, rye and barley. Proceedings of the Royal Society of London B 183, 301-319.

Bennet, M.D., Rao, M.K., Smith, J.B. and Bayliss M.W. 1973b. Cell development in the anther, the ovule and the young seed of *Triticum aestivum* L. var. Chinese Spring. Phil. Transactions of the Royal Society of London B 266, 39-81.

Bruckner, P.L. and Frohberg, R.C. 1987. Rate and duration of grain fill in spring wheat. Crop Sci. 27, 451-455.

Campbell, W.P., Lee, J.W., O'Brien, T.P. and Smart, M.G. 1981. Endosperm morphology and protein body formation in developing wheat grain. Australian Journal of Plant Physiology 8, 5-19.

Cheng, C. and McComb, J.A. 1992. *In vitro* germination of wheat pollen on raffinose medium. New Phytologist 120, 459-462.

Cheng, C. and Rerkasem, B. 1993. Effects of boron on pollen viability in wheat. Plant and Soil 156, 313-315.

Dell, B. 1981. Male sterility and anther wall structure in copper-deficient plants. Annals of Botany 48, 599-608.

El-Ghazaly, G. and Jensen, W.A. 1986a. Studies of the development of wheat (*Triticum aestivum*) pollen: 1. Formation of the pollen aperture. Canadian Journal of Botany 64, 3141-3154.

El-Ghazaly, G. and Jensen, W.A. 1986b. Studies of the development of wheat (*Triticum aestivum*) pollen: 1. Formation of the pollen wall and Ubisch bodies. Grana 25, 1-29.

El-Ghazaly, G. and Jensen, W.A. 1987. Development of wheat (*Triticum aestivum*) pollen: II. Histochemical differentiation of wall and Ubisch bodies during development. American Journal of Botany 74, 1396-1418.

Evans, L.T., Bingham, J. and Roskams, M.A. 1972. The pattern of grain set within ears of wheat. Australian Journal of Biological Sciences 25, 1-8.

Evers, A.D. 1970. Development of the endosperm of wheat. Annals of Botany 34, 547-555.

Gardner, J.S., Hess, W.M. and Trione, E.J. 1985. Development of the young wheat spike: a SEM study of Chinese spring wheat. American Journal of Botany 72, 548-559.

Graham, R.D. 1975. Male sterility in wheat plants deficient in copper. Nature 254, 514-515.

Harrell, D.M., Wilhelm, W.W. and McMaster, G.S. 1993. SCALES - a computer program to convert among 3 developmental stage scales for wheat. Agronomy Journal 85, 758-763.

Hocking, P.J. 1994. Dry-matter production, mineral nutrient concentrations, and nutrient distribution and redistribution in irrigated spring wheat. Journal of Plant Nutrition 17, 1289-1308.

Huber, A.G. and Grabe, D.F. 1987. Endosperm morphogenesis in wheat: transfer of nutrients from the antipodals to the lower endosperm. Crop Science 27, 1248-1252.

Jewell, A.W., Murray, B.G. and Alloway, B.J. 1988. Light and electron microscope studies on pollen development in barley (*Hordeum vulgare* L.) grown under copper-sufficient and deficient conditions. Plant, Cell and Environment 11, 273-281.

Kirby, E.J.M. 1974, Ear development in spring wheat. Journal of Agricultural Science, Cambridge 82, 437-447.

Kirby, E.J.M. and Appleyard, M. 1987. Cereal Development Guide. 2nd edition. Arable Unit, National Agricultural Centre, Stoneleigh, Kenilworth.

Klepper, B. Tucker, T.W. and Dunbar, B.D. 1983. A numerical index to assess early inflorescence development in wheat. Crop Science 23, 206-208.

Kovacs, G., Takacs, I. and Barnabas, B. 1993. In vitro pollen maturation and fertilisation in detached spikelet cultures of wheat. Novenytermeles 42, 119-126.

Kress, W.J. and Stone, D.E. 1983. Pollen intine structure, cytochemistry and function in monocots. In: D.L. Mulcahy and E. Ottaviano (Eds), Pollen: Biology and Implications for Plant Breeding, pp 159-163, Elsevier, NewYork.

Langer, R.H.M. and Hanif, M. 1973. A study of floret development in wheat (*Triticum aestivum* L.). Annals of Botany 37, 743-751.

Large, E.C. 1954. Growth stages in cereals, illustration of the Feekes scale. Plant Pathology 3, 128-129.

Moncur, M.W. 1981. Floral initiation in field crops: an atlas of scanning electron micrographs. CSIRO Melbourne.

Nerson, H., Sibony, M. and Pinthus, M.J. 1980. A scale for the assessment of the developmental stages of wheat (*Triticum aestivum* L.) spike. Annals of Botany 45, 203-204.

Noda, K., Kawabata, C. and Kauzaki, K. 1994. Re-classification of developmental stages of wheat grain. Breeding Science 44, 115-120.

Perry, M. and Hillman, B. 1991. The Wheat Book. A technical manual for wheat producers. Department of Agriculture of Western Australia Bulletin 4196.

Pinthus, M.J. 1985. Triticum. In: A.H. Halevy (Ed.), CRC Handbook of Flowering, Volume 4, pp 418-447.

Rerkasem, B., Saunders, D.A. and Dell, B. 1989. Grain set failure and boron deficiency in wheat in Thailand. Journal of Agriculture (Chiang Mai University) 5, 1-10.

Rerkasem, B., Netsangtip, R., Lordkaew, S. and Cheng, C. 1993. Grain set failure in boron deficient wheat. Plant and Soil 156, 309-312.

Sharma, C.P., Sharma, P.N., Chatterjee, C. and Agarwala, S.C. 1991. Manganese deficiency in maize affects pollen viability. Plant and Soil 138, 139-142.

Sharman, B.C. 1983. Developmental anatomy of the inflorescence of bread wheat (*Triticum aestivum* L.) during normal initiation and when affected by 2, 4-D. Annals of Botany 52, 621-639.

Sibony, M. and Pinthus, M.J. 1988. Floret initiation and development in spring wheat (*Triticum aestivum* L.). Annals of Botany 61, 473-479.

Singh, V.P., Singh, M. and Kairon, M.S. 1984. Physiological maturity in *Aestivum* wheat: visual determination. Journal of Agricultural Science, Cambridge 102, 285-287.

Smart, M.G. and O'Brien, T.P. 1983. Development of the wheat (*Triticum aestivum*) cultivar Heron embryo in relation to neighbouring tissues. Protoplasma 114, 1-13.

Stanley, R.G. and Liskens, H.F. 1974. Pollen, Biology Biochemistry Management. Chapter 8, p 119-128. General Chemistry. Springer-Verlag, Berlin.

Tottman, D.R. and Makepeace, R.J. 1979. An explanation of the decimal code for the growth stages of cereals, with illustrations. Annals of Applied Biology 93, 221-231.

Vishnyakova, M.A. and Willemse, M.T.M. 1994. Pollen pistil interaction in wheat. Acta Botanica Neerlandica 43, 51-64.

Walpole, P.R. and Morgan, D.G. 1970. A quantitative study of grain filling in *Triticum aestivum* L., cultivar Maris Widgeon. Annals of Botany 34, 309-316.

Williams, R.F. 1966. The physiology of growth in the wheat plant. III. Growth of the primary shoot and inflorescence. Australian Journal of Biological Science 19, 949-966.

Whingwiri, E.E., Kuo, J. and Stern, W.R. 1981. The vascular system in the rachis of a wheat ear. Annals of Botany 48, 189-201.□

Zadoks, J.C. Chang, T.T. and Konzak, C.F. 1974. A decimal code for the growth stage of cereals. Weed Research 14, 415-421.